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# CHEMICAL MEDIATION OF APPETITIVE FEEDING IN A MARINE DECAPOD CRUSTACEAN: THE IMPORTANCE OF SUPPRESSION AND SYNERGISM

RICHARD K. ZIMMER-FAUST,*[1] JEFFREY E. TYRE,† WILLIAM C. MICHEL,† AND JAMES F. CASE†

**Department of Zoology, University of Queensland, St. Lucia, Brisbane 4067, Queensland, Australia and †Department of Biological Sciences and the Marine Science Institute University of California Santa Barbara, California, 93106*

## Abstract

The California spiny lobster, *Panulirus interruptus,* failed to exhibit appetitive feeding or locomotion in response to a low molecular weight fraction (<1000 daltons) prepared from a sea water extract of muscle from abalone, a natural prey. This lack of response was caused by chemical suppressants, rather than by lack of stimulatory compounds. Excitatory responses were induced by single, low molecular weight compounds, but these responses were inhibited by suppressants which occur naturally in the muscle fraction. Amino and organic acids were found highly stimulatory to lobsters, but nucleotides and sugars were not. A mixture of monocarboxylic amino acids and dicarboxylic organic acids was much more effective in elliciting behavior than either of the constituents tested alone, at the same overall concentration. Mixtures which combined either ammonium or urea with amino or organic acids significantly reduced behavioral activity caused by these latter substances. Results indicate that tests of single chemicals cannot always reliably predict the stimulatory properties of solutions, combining even as few as two or more compounds. The stimulatory properties of complex odorants, including prey extracts, are best assessed by fractionating and then combining and testing the fractions in bioassays of factorial design.

## Introduction

Behavioral investigations of feeding and electrophysiological studies of chemosensory afference have usually shown that decapod crustaceans are sensitive to low molecular weight compounds. Of these, organic nitrogenous substances and organic acids are the most stimulatory (Laverack, 1963; Case, 1964; McLeese, 1970; Kay, 1971; Shepheard, 1974; Allen *et al.,* 1975; Hindley, 1975; Ache *et al.,* 1978; Johnson and Ache 1978; Mackie *et al.,* 1980; Derby and Atema, 1982a, b). Carbohydrates (Ashby and Larimer, 1965; Hartman and Hartman, 1977; Zimmer *et al.,* 1979; Robertson *et al.,* 1981) and nucleotides (Shelton and Mackie, 1971; Carr and Thompson, 1983) also evoke responses. It is generally assumed that low molecular weight substances are the dominant natural feeding attractants for marine decapods since they stimulate receptors, cause behavioral responses, and are highly soluble and diffusable in sea water (Ache *et al.,* 1976). These latter properties are thought to result in their rapid release from tissues of prey and from carrion (Rittschof, 1980; Zimmer-Faust and Case, 1982a). Because low molecular weight compounds may provide the earliest chemosensory clues to distant food sources, it is generally assumed that decapod predators emphasize their detection in food search and feeding.

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[1] Present address: Marine Science Institute, University of California, Santa Barbara, California, 93106.

Recent electrophysiological investigations have demonstrated that certain low molecular weight substances, abundant in animal flesh, suppress the neural responses of lobster and crab antennule chemoreceptors to stimulatory compounds (Gleeson and Ache, 1983; Johnson and Atema, 1983). While this finding is interesting, there are as yet no clear behavioral correlates for these physiological observations. Suppressants may be found to serve a vital role in the control of foraging and feeding by these animals. For example, suppressants might reduce ingestion of harmful substances, reduce the search for food of low caloric value, or reduce locomotory activity of an animal in the vicinity of a valuable food item, increasing the likelihood of food discovery. Most previous behavioral studies of chemoreception in feeding and food search by decapod crustacea have focused on the role of low molecular weight substances as excitants, typically with tests performed of single chemicals and simple mixtures (McLeese, 1970; Kay, 1971; Allen *et al.,* 1975; Fuzessery and Childress, 1975; Hindley, 1975; Hamner and Hamner, 1977; Hartman and Hartman, 1977; Ache *et al.,* 1978; Robertson *et al.,* 1981; Carter and Steele, 1982; Zimmer-Faust and Case, 1982b). These experiments assumed that summative chemosensory inputs directly control behavior, and little attention has been given to CNS processing. However, in the few studies in which interactions among stimuli were considered synergy was observed (*e.g.,* Shelton and Mackie, 1971; Mackie and Shelton, 1972; Carr, 1978; Robertson *et al.,*1981).

Under field conditions, we have found the California spiny lobster, *Panulirus interruptus* (Randall), arriving in greatest numbers at abalone muscle (*Haliotis* spp.) baits, after 24–48 h (Zimmer-Faust and Case, 1982a). This occurs even though small molecules (primary amines) are released from the baits predominantly over the first 3 h. Moreover, laboratory experiments demonstrate that *P. interruptus* responds significantly to a high molecular weight fraction (>1000 daltons), but not to a low molecular weight fraction (<1000 daltons) prepared from abalone muscle (Zimmer-Faust *et al.,* 1984). Though our field and laboratory observations are in agreement, they differ from what is commonly believed true for decapods, namely, that low molecular weight substances control foraging and feeding. Our results are further at odds with previous electrophysiological investigations which show that *P. interruptus* possesses chemoreceptors sensitive to low molecular weight compounds, occurring abundantly in the flesh of abalone (Fuzessery and Childress, 1975; Lindsey, 1976).

These considerations prompted the present investigation into the behavioral responses of *Panulirus* to low molecular weight substances. Compounds were individually assayed for their ability to stimulate early arousal and appetitive phases of feeding. Some were effective, thus demonstrating specifically for *Panulirus* that inability to respond to the low molecular weight fraction of abalone does not arise from behavioral insensitivity to low molecular weight substances. It was further experimentally demonstrated that this inability was caused by chemical suppressants, and that both synergistic and suppressant interactions occur among substances naturally residing in the tissues of prey and carrion of lobsters. Our results demonstrate that the contributions made by specific chemical agents to the stimulatory capacity of a complex prey extract, cannot be properly specified without considering the entire chemosensory integrative capacity of the responding organism.

## Materials and Methods

### *General procedures*

General procedures and apparatus were identical to those previously described (Zimmer-Faust and Case, 1983; Zimmer-Faust *et al.,* 1984). Animals captured in

traps or by hand (SCUBA) at More Mesa reef, 4 km east of the UCSB campus, were brought immediately to our laboratory and held in 3000 l aquaria for 14 days before experiments were initiated. Incoming animals were tattoo marked for individual recognition (Kuris, 1971), and only hard-shelled animals of 60–68 mm carapace length were used. Animals were fed abalone muscle, mackerel muscle (*Scomber japonicus*), and opened mussels (*Mytilus californianus*) and were deprived of food for 24 h before testing.

Lobsters were individually tested for responses to chemical solutions in rectangular aquaria, 30 × 30 × 13 cm, a size permitting the control of stimulus flow, without inhibiting behavior. Sea water (980 ml/min) entered each aquarium at a velocity of ~50 cm/s, from a head-tank maintained under constant hydrostatic pressure. Stimulants were introduced from a reservoir (10 ml/7 s) by opening a three-way valve. Dilution associated with stimulus delivery was $1.02 \times 10^{-3}$ ($\pm 0.13 \times 10^{-3}$ S.D.) times original concentrations, as previously determined by fluorometric measurement of fluorescein dye dilution (Zimmer-Faust and Case, 1983). Concentrations reported are not corrected for this dilution.

Lobsters were tested once in 48 h for a maximum of 6 times during a 14-day period. They were put in experimental aquaria 90–120 min prior to testing and usually settled within 30–40 min. Observations of behavior were initiated 1 min before introduction of a chemical solution and continued for 3 min afterwards. All trials were conducted according to a double-blind protocol, in which the observer was unaware of the composition of test or control solutions being tested. Order of stimulus presentation did not influence the behavior of animals, since for each substance the proportion responding was unrelated to the test sequence. All solutions were prepared from analytical grade reagents and 5 μm filtered sea water, adjusted to pH 7.8 before testing.

Antennule flicking and wiping, pereiopod probing, and locomotion were monitored, since these are behaviors commonly exhibited by *Panulirus* and other decapods in response to chemicals associated with food (*e.g.,* Maynard and Dingle, 1963; McLeese, 1970; Kay, 1971; Mackie and Shelton, 1972; Snow, 1973; Allen *et al.,* 1975; Fuzessery and Childress, 1975; Hindley, 1975; Pearson and Olla, 1977; Pearson *et al.,* 1979; Zimmer-Faust and Case, 1982b). Appetitive feeding was defined as the occurrence of increased flicking (>1.0 flick/s), wiping and probing each within a 3 min trial period. Further justification for the emphasis on these behavioral acts appears elsewhere (Zimmer-Faust *et al.,* 1984), and their definitions are given in Table I. Chemicals were considered stimulatory when proportions of responding animals differed significantly from the proportion responding to sea water ($P < 0.05$). A G-Test for Independence was used with Williams' correction for 2 × 2 contingency tables, in analyzing data from experiments presenting test solutions to differing groups of animals. A binomial test was used (Sokal and Rohlf, 1981, p. 774; $P = q = 0.5$), for experiments presenting test solutions to the same group of animals, with changes in individual responsivities compared.

*Experiment 1: responses to single compounds*

Previous investigations showed that lobsters are unresponsive to a low molecular weight fraction (<1000 daltons) of an extract prepared from abalone muscle (Zimmer-Faust *et al.,* 1984). For this reason, tests were conducted to determine if lack of response is caused by behavioral insensitivity to low molecular weight substances. Thirty-two compounds were individually assayed at $10^{-2}$ *M,* and each chemical was tested on 20 different animals in conjunction with 40 sea water controls.

TABLE I

*Definitions of behavioral elements in appetitive feeding and locomotion by* Panulirus

| Act | Definition |
|---|---|
| Feeding | |
| Antennule flicking | Vertical deflection of a lateral antennular flagellum to a position nearly contacting the medial flagellum. A response was defined as >1.0 flick per second. |
| Leg probing | Any non-locomotor movement of a pereiopod, either raking a dactyl across the substratum, or elevating a dactyl to a position no longer in contact with the substratum. |
| Antennule wiping | A downward and vertical deflection of an antennule, resulting in simultaneous contact of both antennular flagella with the third maxillipeds. |
| Locomotion | A laterally or anteriorly directed movement of the body to a distance, >1/2 carapace length. |

*Experiment 2: interaction between glycine and the <1000 dalton fraction of freeze-dried abalone muscle extract (FDAME)*

In the first experiment, glycine was found to be the most stimulatory of all tested substances (see Results, Table II). Because a low-molecular weight fraction of FDAME is ineffective in stimulating feeding in *Panulirus,* yet contains a high concentration of glycine ($4.5 \times 10^{-4}$ *M*) (Zimmer-Faust *et al.,* 1984), this finding suggested the existence of suppressants within the fraction. An experiment was conducted to test for this possibility.

A low molecular weight fraction of FDAME was prepared from a standard extract (6.00 g/l) of lyophilized abalone muscle and filtered sea water, by the procedures of Zimmer-Faust *et al.* (1984). Ultrafiltration of the extract was performed using an Amicon model 402 pressure ultrafiltration vessel and UM-2 membrane, with ultrafiltrate (<1000 daltons) collected undiluted, and stored frozen (−20°C) in aliquots. Aliquots of $10^{-4}$ *M* glycine, <1000 fraction with $10^{-4}$ *M* glycine added, and sea water were each presented to the same 27 animals. The application of glycine, by itself, served to control for the possibility that lack of response might be caused by factors other than chemical composition. It was expected that the glycine-enhanced low molecular weight fraction would be ineffective, if suppressants for glycine existed.

*Experiment 3: interactions between glycine and other defined compounds*

Experiments employing the <1000 dalton fraction could not be used to identify the mechanism(s) of feeding suppression, because its constituents might have two types of effects. They might bind to and thereby limit the action of stimulatory molecules, or they might act directly to influence chemosensory processes. Therefore, to approach this question we were constrained to examine the simplest of interactions in this system, namely, those between glycine and other defined compounds. Such tests could demonstrate if suppression directly involves either primary chemosensory processes or CNS mechanisms, by eliminating the possibility of chemical binding or chelating among assayed substances.

*Tests with glycine, urea, and ammonium.* We first explored the interaction between glycine and urea. Urea was selected because it is highly abundant in an extract known

to be noxious to lobsters (J. E. Tyre, upubl. data), prepared from the muscle of angel shark (*Squatina californica*), and because it cannot bind glycine under our present test conditions. Aliquots of $10^{-2}$ *M* glycine, $10^{-2}$ *M* glycine plus $10^{-2}$ *M* urea, $10^{-2}$ *M* urea, and sea water were each presented to the same 52 animals.

Experiments were then performed to investigate the possible interaction between glycine and ammonium. Ammonium was selected because of its close similarity to the major molecular subcomponent of urea, and because of its abundance in the low molecular weight fraction of FDAME ($1.0 \times 10^{-3}$ *M*). Thus, ammonium might serve as a natural suppressant in FDAME to glycine-induced feeding responses. Like urea, it does not bind to glycine under present test conditions. Tests were conducted injecting aliquots of $10^{-2}$ *M* glycine, $10^{-2}$ *M* glycine plus $10^{-2}$ *M* ammonium, $10^{-2}$ *M* ammonium, and sea water, each to the same 32 animals.

Additional tests were performed to further examine the interaction between glycine and ammonium. These tests were conducted by injecting aliquots of $10^{-3}$ *M* ammonium, both by itself, and in combination with $10^{-2}$ and $10^{-4}$ *M* glycine. Tests were also performed by injecting aliquots of $10^{-2}$ and $10^{-4}$ *M* glycine without ammonium added to serve as standards for comparisons with previous trials. Each solution and a sea water control were introduced to the same 20 animals.

*Tests with glycine and taurine.* Another series of tests were performed to investigate the possible interaction between glycine and taurine. Taurine was selected because it is the most abundant free amino acid in FDAME ($6.0 \times 10^{-3}$ *M*), yet is relatively ineffective in causing appetitive feeding responses (see Results, Table II). The structure of taurine differs significantly from those of urea and ammonium, and tests for interactions between taurine and glycine provided a useful comparison. Taurine does not bind glycine under present test conditions. Aliquots of $10^{-2}$ *M* glycine, $10^{-2}$ *M* glycine plus $10^{-2}$ *M* taurine, $10^{-2}$ *M* taurine, and sea water were each presented to the same 20 animals.

*Experiment 4: interactions between succinic acid, urea, and ammonium*

An additional experiment was performed to establish whether any observed suppression by urea and ammonium is specific to stimulations by glycine, or whether urea and ammonium might act more generally to suppress behavior caused by other non-nitrogenous compounds. Succinic acid was selected as an alternative test compound to glycine, because it evokes appetitive responses (see Results, Table II) yet does not possess an amine or amide group as do glycine, urea, and ammonium. Aliquots of $10^{-2}$ *M* succinic acid, $10^{-2}$ *M* succinic acid plus $10^{-2}$ *M* urea, $10^{-2}$ *M* succinic acid plus $10^{-2}$ *M* ammonium, $10^{-2}$ *M* urea, $10^{-2}$ *M* ammonium, and sea water were each presented to the same 32 animals.

*Experiment 5: interactions between amino and organic acids*

Thus far, only those interactions were considered that could possibly lead to a suppression of feeding. Obviously, interactions might also occur to potentiate responses. In the first experiment of this study, testing single compounds, we found at least two major groups of stimulatory molecules to be involved: (1) the small, uncharged monocarboxylic $\alpha$ amino acids and (2) the negatively charged, dicarboxylic organic acids (see Results, Table II). To investigate possible synergistic interactions among these substances, we performed experiments injecting mixtures of equimolar amino acids (glycine, alanine, serine), equimolar organic acids (oxalic, succinic), and equimolar amino and organic acids, each at a total molarity of $10^{-4}$. Each mixture and a sea water (control) were tested on the same 20 animals.

## Results

### *Responses to low molecular weight compounds*

All tested chemicals were detected, as demonstrated by increased rates of antennule flicking (Table II). However, antennule flicking was a poor indicator of overall stim-

Table II

*Appetitive feeding and locomotor responses to single chemicals*[a]

| Compound[b] | Feeding component | | | |
|---|---|---|---|---|
| | Antennule flicking (Detection) | Pereiopod probing | Antennule wiping | Locomotion |
| Amino acids (L-isomers) | | | | |
| glycine | 1.00*** | 0.95*** | 0.75*** | 0.15 |
| alanine | 1.00*** | 0.75** | 0.65*** | 0.05 |
| serine | 1.00*** | 0.70** | 0.60*** | 0.15 |
| methionine | 1.00*** | 0.70** | 0.40** | 0.10 |
| isoleucine | 1.00*** | 0.65* | 0.35* | 0.05 |
| leucine | 1.00*** | 0.70** | 0.30* | 0.10 |
| glutamic acid | 1.00*** | 0.65* | 0.30* | 0.15 |
| valine | 1.00*** | 0.55 | 0.30* | 0.10 |
| threonine | 1.00*** | 0.40 | 0.30* | 0.00 |
| histidine | 0.95*** | 0.40 | 0.30* | 0.00 |
| lysine | 1.00*** | 0.60* | 0.25* | 0.20 |
| phenylalanine | 1.00*** | 0.45 | 0.25* | 0.00 |
| aspartic acid | 0.95*** | 0.35 | 0.05 | 0.00 |
| arginine | 0.85** | 0.35 | 0.05 | 0.00 |
| taurine | 1.00*** | 0.70** | 0.00 | 0.05 |
| Organic acids (L-isomers) | | | | |
| succinic | 1.00*** | 0.65* | 0.55*** | 0.30* |
| malic | 1.00*** | 0.30 | 0.15 | 0.15 |
| ascorbic | 0.85** | 0.35 | 0.10 | 0.05 |
| citric | 0.95*** | 0.30 | 0.10 | 0.05 |
| oxalic | 1.00*** | 0.60* | 0.05 | 0.35* |
| propionic | 0.80* | 0.35 | 0.00 | 0.05 |
| Carbohydrates (D-isomers) | | | | |
| glucose | 0.95*** | 0.65* | 0.10 | 0.05 |
| mannose | 1.00*** | 0.45 | 0.05 | 0.15 |
| fructose | 0.90** | 0.45 | 0.05 | 0.20 |
| maltose | 1.00*** | 0.40 | 0.00 | 0.10 |
| Nucleotides (5′-monophosphates) | | | | |
| cytosine (CMP) | 0.95*** | 0.35 | 0.00 | 0.15 |
| guanine (GMP) | 0.95*** | 0.20 | 0.00 | 0.00 |
| adenine (AMP) | 0.90** | 0.20 | 0.00 | 0.05 |
| Miscellaneous | | | | |
| betaine | 0.95*** | 0.75** | 0.35* | 0.15 |
| trimethylamine | 0.90** | 0.60* | 0.15 | 0.15 |
| glutathione (reduced) | 0.85** | 0.45 | 0.15 | 0.00 |
| ammonium | 1.00*** | 0.70** | 0.10 | 0.00 |
| Sea water (controls) | 0.45 | 0.33 | 0.05 | 0.10 |

[a] Data are expressed as proportions of responding animals.

[b] Each compound was tested on 20 different animals at an injected concentration of $10^{-2}$ *M*. Sea water was tested on 40 different animals.

* The difference between the proportion of animals responding to test *versus* sea water (control) solutions is significant (G-Test and Williams' Correction: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

ulatory capacity, since the activation of feeding sequences varied tremendously among the detected substances. Of the 15 amino acids tested, only 8 induced all three of the appetitive behaviors, with glycine, alanine, and serine being the most effective (Table II). Alpha amino acids were ranked by their ability to effect appetitive feeding and the ranked order was found to be inversely correlated with molecular weight (Kendall's Tau: $\tau = -0.64$, $P < 0.01$, n = 14). Alpha amino acids having uncharged R-groups were also more effective than charged molecules in activating appetitive behavior (Mann-Whitney U Test: $T_X = 20$, $P = 0.009$, n = 9, m = 5); thus, both molecular weight and charge contributed significantly to the stimulatory capacity of these substances. Glycine initiated appetitive feeding at concentrations of $10^{-2}$ and $10^{-4}$, but not at $3.33 \times 10^{-5}$ or $10^{-6}$ *M* (Fig. 1). Appetitive feeding did not occur at any test concentration of taurine, though increased antennule flicking and pereiopod probing were observed at the highest concentration. Succinic acid and betaine were the only other chemicals to initiate all three of the appetitive behaviors, while sugars and nucleotides were generally ineffective as stimulants. Only two compounds, oxalic and succinic acids, caused significant locomotor responses (Table II).

*Interaction between glycine and the <1000 dalton fraction of FDAME*

Responses to the glycine-enhanced, low molecular weight fraction were not significantly different from those to sea water, for any assayed behavior (Table III). Significance was approached in the case of antennule flicking, showing that the en-

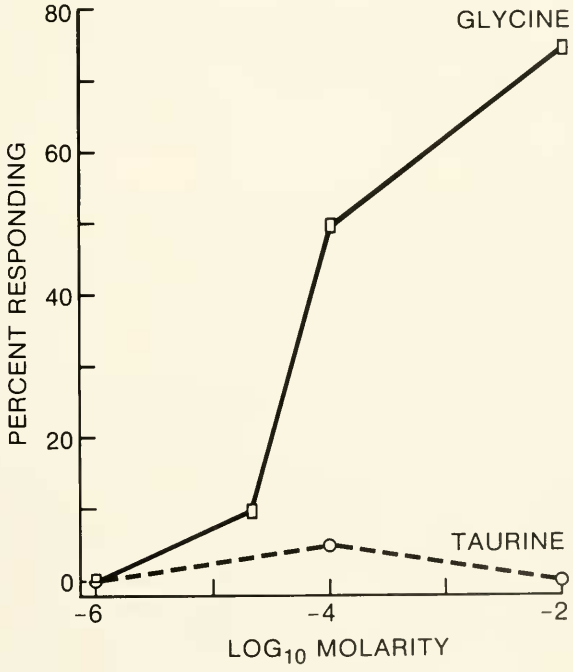


FIGURE 1. Proportions of animals showing appetitive feeding responses to glycine and to taurine. Glycine was an effective stimulant at $10^{-2}$ and $10^{-4}$ *M*, while taurine was ineffective at all tested concentrations. Five percent of all animals responded to sea water controls. Appetitive feeding was defined as the occurrence of probing, wiping and increased flicking, each within a 3-min trial period.

TABLE III

*Interaction between glycine and the <1000 dalton fraction of FDAME*[a]

| Test solution | Injected glycine concentration ($M$) | | Feeding component: Antennule flicking (detection) | Pereiopod probing | Antennule wiping | Loco-motion |
|---|---|---|---|---|---|---|
| glycine | | $1.0 \times 10^{-4}$ | 0.96* | 0.78** | 0.48** | 0.09 |
| <1000 dalton fraction | $4.5 \times 10^{-4}$ | $5.5 \times 10^{-4}$ | | | | |
| glycine | $1.0 \times 10^{-4}$ | | 0.93 | 0.56 | 0.22 | 0.06 |
| sea water | | —— | 0.78 | 0.44 | 0.15 | 0.06 |

[a] Data are expressed as proportions of responding animals, and n = 27.

* The difference between the proportion of animals responding to test *versus* sea water (control) solutions is significant (Binomial Test: $*P < 0.05$, $**P < 0.01$).

hanced fraction was probably detected by lobsters, but was otherwise ineffective as a stimulant. The $10^{-4}$ $M$ glycine (control) solution caused significant responses, relative to sea water, demonstrating that response failure of the glycine-enhanced low molecular weight fraction was not caused by factors other than chemical composition. Responses to glycine were compared with those to the glycine-enhanced fraction, and significant differences were found for pereiopod probing (Binomial Test: k = 6, Y = 6, $P = 0.02$) and for antennule wiping (k = 10; Y = 9, $P = 0.01$). This clearly demonstrated an existence of suppressants in the low molecular weight fraction, since the enhanced fraction contained 5.5× more glycine stimulant than the control ($10^{-4}$ $M$) solution. These results are even more impressive when it is considered that glycine was only one of several known stimulatory compounds in the low molecular weight fraction, which includes alanine and glutamic acid among others (Zimmer-Faust *et al.*, 1984).

*Interaction between glycine, urea, and ammonium*

Both urea and ammonium acted as mild stimulants when tested at $10^{-2}$ $M$, inducing flicking and probing but not wiping or locomotion (Table IV, Expts. A and B). Glycine evoked all four assayed behaviors at $10^{-2}$ $M$, but did not initiate locomotor responses at $10^{-4}$ $M$. Responses to glycine differed significantly from those to urea-glycine, in pereiopod probing (Binomial Test: $P < 0.005$), antennule wiping ($P < 0.01$) and locomotion ($P = 0.01$), which showed that urea was inhibitory to the glycine response (Table IV, Expt. A). Glycine-induced wiping responses were inhibited by ammonium, when ammonium ($10^{-2}$ and $10^{-3}$ $M$) was combined with either a high ($10^{-2}$ $M$) or low ($10^{-4}$ $M$) concentration of glycine (Table IV, Expts. B and C and Binomial test: $P < 0.01$, all comparisons). Ammonium was also inhibitory to probing and to locomotion when combined at $10^{-2}$ or $10^{-3}$ $M$ with a high concentration ($10^{-2}$ $M$) of glycine ($P < 0.05$, all comparisons). Results identified at least one inhibitory interaction (ammonium-glycine) that could affect the stimulatory capacity of FDAME. It is of major interest that both urea and ammonium were by themselves slightly stimulatory, since this means that not all combinations of substances which are stimulants when presented singly necessarily heighten behavioral responses.

*Interaction between glycine and taurine*

Proportions of animals responding to glycine and to taurine were almost identical to those of previous experiments (Table IV, Experiment D). Glycine evoked significant

TABLE IV

*Interactions between glycine and other defined compounds*[a]

| Experiment | Test solution | Injected concentration | | Feeding component: Antennule flicking (detection) | Pereiopod probing | Antennule wiping | Locomotion |
|---|---|---|---|---|---|---|---|
| A | glycine | | $1.0 \times 10^{-2}$ | 1.00*** | 0.77*** | 0.63*** | 0.21* |
| (n = 52) | glycine + urea | $1.0 \times 10^{-2}$ + $1.0 \times 10^{-2}$ | $2.0 \times 10^{-2}$ | 1.00*** | 0.58** | 0.36* | 0.08 |
| | urea | | $1.0 \times 10^{-2}$ | 1.00*** | 0.48** | 0.04 | 0.08 |
| | sea water | | —— | 0.35 | 0.21 | 0.04 | 0.04 |
| B | glycine | | $1.0 \times 10^{-2}$ | 1.00*** | 0.78*** | 0.66*** | 0.22* |
| (n = 32) | glycine + ammonium | $1.0 \times 10^{-2}$ + $1.0 \times 10^{-2}$ | $2.0 \times 10^{-2}$ | 1.00*** | 0.47** | 0.34* | 0.06 |
| | ammonium | | $1.0 \times 10^{-2}$ | 1.00*** | 0.56** | 0.00 | 0.06 |
| | sea water | | —— | 0.34 | 0.19 | 0.06 | 0.06 |
| C | glycine | | $1.0 \times 10^{-2}$ | 1.00*** | 0.95*** | 0.80*** | 0.20 |
| (n = 20) | | | $1.0 \times 10^{-4}$ | 1.00*** | 0.75*** | 0.55*** | 0.05 |
| | glycine + ammonium | $1.0 \times 10^{-2}$ + $1.0 \times 10^{-3}$ | $1.1 \times 10^{-2}$ | 1.00*** | 0.75*** | 0.40** | 0.00 |
| | | $1.0 \times 10^{-4}$ + $1.0 \times 10^{-3}$ | $1.1 \times 10^{-3}$ | 1.00*** | 0.85*** | 0.20 | 0.00 |
| | ammonium | | $1.0 \times 10^{-3}$ | 1.00*** | 0.55 | 0.00 | 0.00 |
| | sea water | | —— | 0.40 | 0.35 | 0.05 | 0.05 |
| D | glycine | | $1.0 \times 10^{-2}$ | 1.00*** | 0.75*** | 0.70*** | 0.20 |
| (n = 20) | glycine + taurine | $1.0 \times 10^{-2}$ + $1.0 \times 10^{-2}$ | $2.0 \times 10^{-2}$ | 1.00*** | 0.80*** | 0.65*** | 0.20 |
| | taurine | | $1.0 \times 10^{-2}$ | 1.00*** | 0.70*** | 0.00 | 0.10 |
| | sea water | | —— | 0.45 | 0.40 | 0.05 | 0.10 |

[a] Data are expressed as proportions of responding animals.

* The difference between the proportion of animals responding to test *versus* sea water (control) solutions is significant (Binomial Test: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

flicking, probing, and wiping responses, but not locomotion. Non-significant changes in locomotor behavior resulted more from a slightly elevated response to sea water (controls) and from a smaller sample size, than from a reduced responsivity to glycine. Taurine significantly induced flicking and probing, but not wiping or locomotion. Responses to the taurine-glycine solution were exhibited by nearly equal proportions of animals, as were those to the glycine (control) solution, and no significant differences were found (Table IV, Expt. D). This showed that taurine has no effect on stimulation by glycine, which is important, since it clearly demonstrates that suppression is dependent on the nature of the interacting compounds, in our test system.

*Interaction between succinic acid, urea, and ammonium*

Urea and ammonium evoked flicking and probing, while succinic acid induced each of the four assayed behaviors (Table V). Comparisons of responses to succinic acid and to interactive solutions (urea-succinic acid and ammonium-succinic acid) identified significant differences in antennule wiping (Binomial Test: $P \leq 0.03$, both

TABLE V

*Interactions between succinic acid and other defined compounds*[a]

| Test solution | Injected concentration ($M$) | | Antennule flicking (detection) | Pereiopod probing | Antennule wiping | Locomotion |
|---|---|---|---|---|---|---|
| succinic acid | | $1.0 \times 10^{-2}$ | 0.97*** | 0.63** | 0.38*** | 0.28** |
| succinic acid + urea | $1.0 \times 10^{-2}$ + $1.0 \times 10^{-2}$ | $2.0 \times 10^{-2}$ | 0.94*** | 0.50** | 0.16 | 0.06 |
| succinic acid + ammonium | $1.0 \times 10^{-2}$ + $1.0 \times 10^{-2}$ | $2.0 \times 10^{-2}$ | 1.00*** | 0.50** | 0.06 | 0.13 |
| urea | | $1.0 \times 10^{-2}$ | 1.00*** | 0.53** | 0.06 | 0.09 |
| ammonium | | $1.0 \times 10^{-2}$ | 1.00*** | 0.56** | 0.00 | 0.06 |
| sea water | | — | 0.34 | 0.19 | 0.06 | 0.06 |

[a] Data are expressed as proportions of responding animals, and n = 32.

* The difference between the proportion of animals responding to test *versus* sea water (control) solutions is significant (Binomial Test: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

comparisons) and locomotion ($P \leq 0.03$, both comparisons). Thus, both urea and ammonium are inhibitory to succinic acid, as well as to glycine.

*Interaction between amino and organic acids*

Amino and organic acids were more effective when tested as combined stimuli, than when tested singly. This was demonstrated by our finding that the mixture of amino acids and the mixture of organic acids were only slightly stimulatory, at the tested concentration ($10^{-4}$ $M$), while a mixture combining these substances was highly effective (Table VI). Data showed that an increase in responsivity by test animals

TABLE VI

*Interaction between amino and organic acids*[a]

| Test solution | Injected concentration ($M$) | | Feeding Component: Antennule flicking (detection) | Pereiopod probing | Antennule wiping | Locomotion |
|---|---|---|---|---|---|---|
| glycine, alanine, serine | $3.33 \times 10^{-5}$ each | $1.00 \times 10^{-4}$ | 0.95*** | 0.40 | 0.10 | 0.15 |
| succinic acid, oxalic acid | $5.00 \times 10^{-5}$ each | $1.00 \times 10^{-4}$ | 0.75* | 0.30 | 0.00 | 0.05 |
| glycine, alanine, serine, succinic acid, oxalic acid | $2.00 \times 10^{-5}$ each | $1.00 \times 10^{-4}$ | 0.90** | 0.45 | 0.40*** | 0.30* |
| sea water (controls) | | —— | 0.40 | 0.35 | 0.00 | 0.00 |

[a] Data are expressed as proportions of responding animals, and n = 20.

* The difference between the proportion of animals responding to test *versus* sea water (control) solutions is significant (Binomial Test: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

was the result of differences in the qualitative chemical composition of mixtures, because overall chemical concentration was maintained constant. Proportions of animals initiating wiping and locomotion were actually greater in response to the combined amino-organic acid mixture, than those in response to the amino acid mixture added with those in response to the organic acid mixture. The interaction between amino and organic acids was clearly synergistic.

## Discussion

Our results show that low molecular weight compounds induce both appetitive feeding and locomotion in the California spiny lobster, *Panulirus interruptus.* This is noteworthy, because it differs from our previous findings with *P. interruptus,* in which a low molecular weight fraction (<1000 daltons) of abalone muscle was unable to stimulate appetitive feeding in laboratory tests (Zimmer-Faust *et al.,* 1984), and also failed to attract lobsters to baited traps in field experiments (Zimmer-Faust and Case, 1982a). Apparent disparity in our findings is not the result of differences in experimental procedures, since these were the same for all laboratory tests, nor is it the result of a lack of stimulatory substances within the low molecular weight fraction. Several compounds occurring in the fraction were found to be stimulatory when presented individually to *P. interruptus.* The failure of the low molecular weight fraction must, therefore, be attributed to a presence of substances that either affect the chemical senses of lobsters, or bind to, and thereby limit the action of, stimulatory molecules. In distinguishing between these possibilities, we first performed an experiment to directly demonstrate that feeding suppression is caused by chemicals residing in the low molecular weight fraction of abalone. Suppression was then determined to affect the chemosensory processes of lobsters in experiments showing ammonium and urea inhibiting the behavior caused by glycine and succinic acid, without binding these substances.

Synergism in the stimulation of lobsters was observed upon combining monocarboxylic amino acids, including glycine, with dicarboxylic organic acids, including succinic acid. Synergism is generally recognized to arise, at least in part, from the simultaneous stimulation of different chemoreceptor sites, each varying in chemical specificity (*e.g.,* Shelton and Mackie, 1971; Mackie and Shelton, 1972; Dethier, 1976; Kroeze, 1981). Our results suggest that glycine and succinic acid may be stimulating different receptor sites and possibly different cell populations. Because both urea and ammonium were inhibitory to glycine and to succinic acid, it is further suggested that (1) urea and ammonium may act non-specifically to suppress behavior caused by afferences from at least two different receptor sites, and (2) competitive interactions between suppressants (urea and ammonium) and stimulants seem unlikely to occur, at both receptor sites for glycine and for succinic acid, due to major differences in the molecular structures and charges of these substances.

Neurophysiological experiments are now needed to test these hypotheses. Data are available from at least one neurophysiological study of chemoreceptors on the antennules of *Panulirus interruptus,* and these generally support our findings. In this investigation, two classes of chemoreceptors were identified. One responded predominantly to mono-, and the other responded to di-carboxylic amino acids (Fuzessery and Childress, 1975). It was postulated in that study that through stimulation of receptors having different specificities, a mixture of mono- and di-carboxylic amino acids might heighten feeding responses, presumably through a CNS mechanism. Results of present behavioral experiments are very similar to those postulated, though we did substitute dicarboxylic organic acids for their amino acid counterparts.

It is well known that suppressants occur in the tissues of a variety of plants and

animals, to be used in chemical defenses against predation (*e.g.*, see Sondheimer and Simeone, 1970; Chapman, 1974; Kittredge *et al.*, 1974; Shorey and McKelvey, 1977; Faulkner and Ghiselin, 1983 for reviews). In the present study, however, we observed abalone muscle to contain suppressants, yet it is readily consumed by lobsters. Furthermore, abalone muscle causes appetitive feeding responses in lobsters (Zimmer-Faust *et al.*, 1984), and it is highly attractive to lobsters when used as a bait (Zimmer-Faust and Case, 1982a). This demonstrates that suppressants are not in this circumstance operating strictly in chemical defense. We consider an alternative, namely, that sensitivities to suppressants are employed by lobsters to enhance discriminatory abilities. It has been clearly demonstrated for both insects and for vertebrates, that sensitivities to stimulants and to suppressants gives greater control over the modulation and tuning of feeding, according to overall quality of a chemical mixture or food odor (*e.g.*, Dethier, 1966, 1976; Fishman, 1971; Shumake *et al.*, 1971; Harborne, 1982a, b; Thompson *et al.*, 1983).

The paradox that lobsters evidently should invoke and also over-ride suppressant sensitivities, as in the case of abalone muscle, is easily resolved by considering that suppressants may be widespread. They may simply occur either in greater abundances or in an absence of stimulants in tissues of non-preferred foods. Our findings with ammonium and urea support such a conclusion. Each is a major product of nitrogen catabolism and is widespread in tissues of marine invertebrates (Campbell, 1973). Strict avoidance of these substances is impossible by feeding lobsters, though we have found lobsters avoiding the cephalothoraces of crustacean prey and carrion, tissues which accumulate and excrete ammonium and urea. Ammonium is produced in copious amounts by both anaerobic and aerobic decomposing bacteria (*e.g.*, Kjosbakken *et al.*, 1983), and it is non-nutritive to decapod crustacea. Avoidance and reduced ingestion of this compound would seem beneficial, particularly if not associated with ingestion of high quality, nutritive substances.

It must be questioned why ammonium and urea suppress stimulation by other compounds, yet are slightly stimulatory by themselves. This is best explained by the finding that antennule flicking (Snow, 1973; Pearson and Olla, 1977; Price and Ache, 1977; Pearson *et al.*, 1979; Schmitt and Ache, 1979; present study) and pereiopod movements (Atema and Engstrom, 1971) are in part associated with a generalized alerting response of decapods to chemical substances, and it is these behaviors that are evoked by ammonium and urea. Flicking and probing contrast sharply to antennule wiping, a behavior exhibited by *P. interruptus* predominantly in response to food-related chemical stimuli (J. E. Tyre, unpubl. data), and not induced by either ammonium or urea. It has been suggested by previous investigators that ammonium acts as a major feeding stimulant to the American lobster, *Homarus americanus* (Carter and Steele, 1982; Derby and Atema, 1982a). While this may be true, we point out that ammonium is released by invertebrate and vertebrate *predators* of lobsters, by bacterial decomposers, and by lobsters themselves, as well as by invertebrate prey species.

Our identification of suppressants raises several interesting questions concerning experimental methods in investigations of crustacean chemoreceptive and feeding behavior. In particular, studies which rely on tests of single compounds may not properly define the natural responsivities of test animals. This is suggested by our finding that single, low molecular weight substances, such as glycine, initiate behavioral responses when presented by themselves, but do not always contribute to the stimulatory capacity of complex, prey extracts. Furthermore, chemicals that are slightly stimulatory by themselves (*e.g.*, ammonium and urea) can profoundly reduce the stimulatory capacity of even simple mixtures.

Further difficulties arise upon considering investigations based on extracts of natural tissues and component fractions. These studies may incorrectly specify the potencies of component fractions, unless each fraction is tested both by itself and in all possible combinations. This is because potency is often non-additive, and the stimulatory capacity of a specific compound or fraction cannot always be predicted from those of others. For example, we have found FDAME to contain glycine at $5.0 \times 10^{-4}$ *M* and to induce appetitive feeding in 40% of all tested animals (Zimmer-Faust *et al.*, 1984). A five-fold dilution of glycine ($1.0 \times 10^{-4}$ *M*), tested by itself, was found to induce appetitive feeding in a slightly higher proportion of animals (50%). It might be concluded incorrectly from these results that glycine alone is responsible for all activity generated by whole extract (FDAME). This we know is untrue, since the <1000 dalton fraction of FDAME is unstimulatory.

Our results for *Panulirus* indicate that, if compounds and fractions are to be properly identified for their contributions to the stimulatory capacity of prey odors, then experiments must be performed to assess the interactions which occur among the stimuli. This can best be accomplished by using bioassays in which fractions are prepared and tested both by themselves and in combination, in experiments of either factorial or combinatorial design. This way, interactions between chemical components can be identified for their influences on behavior by analyzing the responses of test animals in a multiway analysis of variance, where each fraction is treated as an independent factor (Sokal and Rohlf, 1981), or by comparing the deviation of response values from those predicted by derivations of simple, additive and non-additive models. The exact nature of each interaction can then be described according to its synergistic or additive properties, and according to its facilitative or inhibitory effect on behavior.

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